IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cantor *et al.*

Serial No.: 09/395,409

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For: *SOLID PHASE SEQUENCING OF BIOPOLYMERS*

Art Unit: 1656

Examiner: Houtteman, S.

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12/19/01
Date

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MARKED UP CLAIMS (37 C.F.R. § 1.121)

Please amend claims 77, 86, 88 and 89 as follows (insertions are underlined, deletions are [bracketed]):

77. (Amended) A method of detecting a target nucleic acid, comprising the steps of:

providing a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid;

hybridizing the set to an array of nucleic acid probes to form a target array of nucleic acids, wherein each probe comprises a single-stranded portion comprising a variable region; and

determining molecular weights for nucleic acids of the target array;

whereby the [sequence of the] target nucleic acid is [determined] detected.

86. (Amended) The system of claim 127 [85], wherein the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete diversity or nearly complete diversity.

88. (Amended) The method of claim [56] 128, wherein the molecular weights are determined by methods selected from the group consisting of gel electrophoresis, capillary electrophoresis, chromatography, and nuclear magnetic resonance.

89. (Amended) The method of claim [56] 128, wherein the molecular weights are determined by mass spectrometry.

THE REJECTION OF CLAIMS 88-127 UNDER 37 CFR 1.142(b)

Claims 88-127 are rejected as being drawn to subject matter that is independent or distinct, and hence restrictable, from the invention originally claimed. It is asserted in the Office Action that claims 88-127 are drawn to newly claimed **methods** of detection comprising gel electrophoresis, capillary electrophoresis, chromatography, nuclear magnetic resonance, mass spectrometry and to probe **arrays** comprising 4^R probes with a variable region of length R and single-stranded portions and double-stranded portions. While claims 88-123 are directed to methods, they are not directed to methods of "detection" as stated in the Office Action, but instead are directed to methods of **sequencing** a target nucleic acid and further specify techniques whereby the molecular weight determination step of former claim 56 (which has been added back to the claims in its original form as new claim 128), which depends from claim 1, may be accomplished. It is further noted that claim 127 is directed to a **system** (not a method or array) which is nearly identical to the system of originally presented claim 87, which is cancelled herein, except that the number of probes in the probe array of the system is specified in claim 127.

The Examiner urges that claims 88-127 submitted by Amendment on April 6, 2001 are related to the pending claims as a subcombination thereof that is restrictable from the original claims. In view of this interpretation, the Examiner has withdrawn claims 88-127 from consideration as being directed to non-elected subject matter.

The molecular weight determination methods recited in claims 88 and 89 are identical to those recited in claims 2 and 3, respectively. Claims 88 and 89 were dependent on previous claim 56 (now claim 128) which is dependent on claim 1. Thus, the methods of claims 88 and 89 differ from the methods of claims 2 and 3 only in that they specify that the probes of the methods of claims 88 and 89 contain a double-stranded portion as well as a single-stranded portion (as specified in former claim 56 now claim 128). If claims 88-127 are restricted from all the

other pending claims, applicant ultimately could be granted two patents, one patent which includes a claim directed to a sequencing method with steps of hybridizing a set of target nucleic acid fragments to an array of probes comprising a single-stranded portion containing a variable region and a double-stranded portion to form a target array and determining the molecular weights of nucleic acids of the target array, and a second patent that includes claims directed to the same method in which the molecular weights are determined by a particular molecular weight determination method. By virtue of the restriction requirement, such patents will not be required to be co-owned and could expire on different dates. Thus, for example, if the first patent suggested above issues first, a later issuing patent such as the second patent suggested above could not be held to constitute obvious-type double patenting over the earlier issued patent. See MPEP 806, paragraph 3, which states:

> [w]here inventions are related as disclosed but are not distinct as claimed, restriction is never proper. Since, if restriction is required by the Office double patenting cannot be held, it is imperative the requirement should never be made where related inventions as claimed are not distinct.

See, also MPEP 804.01, which states:

> 35 U.S.C.121, third sentence, provides that wherein the Office requires restriction, the patent of either the parent or any divisional application thereof conforming to the requirement cannot be used as a reference against the other. This apparent nullification of double patenting as ground of rejection or invalidity in such cases imposes a heavy burden on the Office to guard against erroneous requirements for restriction where the claims define essentially the same inventions in different language and which, if acquiesced in, might result in the issuance of several patents for the same invention.

THE REJECTION OF CLAIMS 1-55, 58-60, 63-77, and 86 UNDER 35 U.S.C. §103

Claims 1-55, 58-60, 63-77, 86 and 87 are rejected under 35 U.S.C. §103(a) as being unpatentable over Khrapko *et al.* (J. DNA Sequencing and Mapping 1: 375-388) in view of Drmanac *et al.* (DNA and Cell Biology 9: 527-534). As

pointed out in the Response (mailed April 4, 2001) to the previous Office Action, claims 1-55, 58-60, and 63-77, which are directed to methods of sequencing or detecting a target nucleic acid, each require the determination of the molecular weights of the nucleic acids of a target array. As urged in the previous Response, neither Khrapko *et al.* nor Drmanac *et al.*, singly or in any combination thereof, teaches or suggests a method of sequencing that includes a step in which the molecular weight of nucleic acids in a target array are determined.

In the current Office Action, it is asserted that Applicant's previous arguments in support of patentability of the rejected claims are not persuasive. Specifically, it is alleged that because the prior art teach methods of nucleic acid sequencing, and all sequencing methods involve first a fragmentation step and then a method of determining the molecular weight of the fragment, the molecular weight step is implicit in any prior art method of nucleic acid sequencing.

The rejection is respectfully traversed.

Relevant law

Under 35 U.S.C. §103, in order to set forth a case of *prima facie* obviousness, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. *See, e.g., Lindemnann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1462, 221 U.S.P.Q.2d 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); *In re Papesh*, 315 F.2d 381, 137.U.S.P.Q. 43 (CCPA 1963).

The Claims

Claims 1-55, 58-60, and 63-77 are directed to methods of sequencing or detecting a target nucleic acid molecule by hybridizing nucleic acid fragments containing a sequence that corresponds to a sequence of the target nucleic acid to

an array of nucleic acid probes that contain a single-stranded portion comprising a variable region to form a target array and then determining the molecular weights of nucleic acids of the target array. Claim 128 and claims 88 and 89, and claims dependent thereon, recite that the probes include a double-stranded region.

Claims 86 and 87 are directed to a system containing a mass spectrometer, a computer and an array of 4^R nucleic acid probes comprising a double-stranded portion and a single-stranded portion which contains a variable sequence of length R wherein the array is attached to a solid support comprising a matrix that facilitates volatilization of nucleic acids for mass spectrometry.

Differences between the cited references and the claimed subject matter

Khrapko *et al.*

Khrapko *et al.* describes a technique of DNA sequencing by hybridization with an oligonucleotide matrix (SHOM) and experiments to test the method on a short (17 nucleotides) DNA fragment. The method relies upon hybridization of **labeled** fragments of a target sequence to a set of 65,536 (4^8) oligomers of 8 nucleotides long, which constitute all possible combinations of 8-mers. The sequence of a particular target can be resolved by identifying the oligonucleotides to which it hybridizes and comparing overlaps among the hybridizing oligomers. The sequence of a target nucleic acid may be ascertained by analyzing the pattern produced by the labeled hybrids.

The method is stated to require a determination of the dissociation curve for all hybridizing oligonucleotides in order to differentiate perfect duplexes from imperfect duplexes (i.e., containing base pair mismatches). Comparison of temperature-dependent dissociation curves of the duplexes formed by DNA and each of the immobilized oligonucleotides, with standard dissociation curves for perfect oligonucleotide duplexes to thereby identify perfect duplexes and/or the degree of mismatch. The duplexes were subjected to a series of washes at increasing temperatures, and thermal dissociation curves were generated and compared to distinguish perfect from imperfect hybrids to thereby identify the

perfectly matched hybrids from which to deduce the sequence of the target.

Khrapko *et al.* suggests that additional continuous stacking hybridization (CSH), referred to as hybridization of DNA with immobilized octanucleotides **in the presence of** labeled selected pentanucleotides to form a continuously stacked perfect duplex of 13 base pairs, could increase the fidelity of SHOM (emphasis added, see page 376, first full paragraph in left column). Additional experiments reviewed in Khrapko *et al.* include a "numerical" experiment to estimate the efficiency of CSH. In the description of CSH (p. 385, first full paragraph in left column), Khrapko *et al.* states that it is based on the fact that when two oligonucleotides are **simultaneously** hybridized to a longer one, the two duplexes are mutually stabilized if they are positioned side-by-side due to a stacking contact between them. Figure 8 of Khrapko *et al.* is said to illustrate this effect.

Khrapko describes that sequencing is effected by identifying hybrids by detecting the labeled oligonucleotide and conducting overlapping block reading of the oligonucleotide sequences to which the target nucleic acid hybridizes in order to reconstruct the target sequence. This method is described on page 375 (first full paragraph in the right column) where it is stated "[t]o illustrate Sequencing by Hybridization to Oligonucleotide Matrix (SHOM), let us take the simple example of a labeled fragment CTCA (TGAG as a complementary strand) and a matrix of the whole set of $4^3 = 64$ trinucleotides. The fragment will specifically hybridize only with complementary trinucleotides TGA and GAG revealing the presence of these blocks in the complementary sequence. Overlapping between the trinucleotides TGA and GAG by the dinucleotide GA enables one to reconstruct the initial tetranucleotide TGAG." There is no affirmative or implicit or even accidental step in the method described in Khrapko of determining the molecular weight of any nucleic acid.

Furthermore, Khrapko does not teach or suggest such a method of sequencing a target nucleic acid that includes a step of determining the molecular weights for nucleic acids of a target-probe hybrid by using mass spectrometry

(claim 3 and dependents) or gel electrophoresis, capillary electrophoresis, chromatography, or nuclear magnetic resonance (claim 2). Khrapko does not teach or suggest such a method of sequencing a target nucleic acid that includes a step of enzymatically extending nucleic acid probes of an array using hybridized target nucleic acid as a template to form extended strands (claim 9 and dependents). Khrapko does not teach or suggest such a method of sequencing a target nucleic acid that includes hybridization of target nucleic acid to an array of nucleic acid probes having at least one mass-modifying functionality (claim 12 and dependents).

Khrapko does not teach or suggest such a method of sequencing that includes a step of removing alkali cations (claim 31 and dependents) or dephosphorylating the nucleic acid fragments by treatment with a phosphatase prior to hybridization (claim 42). Khrapko does not teach or suggest such a method of sequencing that includes a step of producing nucleic acid fragments by enzymatically digesting the target nucleic acid (claim 43), physically cleaving the target nucleic acid (claim 45), by enzymatic polymerization using the target nucleic acid as a template (claim 46), or by synthesizing a complementary copy of the target sequence (claim 49). Khrapko does not teach or suggest such a method of sequencing in which an array of nucleic acid probes is attached to a solid support that includes a matrix that facilitates volatilization of nucleic acids for molecular weight determination (claim 75). Khrapko also does not teach or suggest a method of detecting a target nucleic acid that includes a step of determining the molecular weight of any nucleic acid and thus does not teach or suggest the method of claim 77.

Furthermore, Khrapko does not teach or suggest an array of nucleic acid probes comprising a single-stranded portion and a double-stranded portion, and each single-stranded portion comprises a variable sequence, and the collection contains 4^R probes where R is the length of the variable region (claim 124); or such an array attached to a solid support comprising a matrix that facilitates volatilization (claims 125 and 126).

Drmanac *et al.*

Drmanac describes experiments designed to investigate possible DNA hybridization conditions that may permit discrimination between perfectly matched duplexes and duplexes with a single mismatch. In these experiments, single-stranded DNA was spotted on a membrane and then hybridized with an oligomer probe end-labeled with ^{32}P. Autoradiographs of the membranes and liquid scintillation counting methods were utilized to demonstrate hybridization.

Experiments with model oligonucleotides and an M13 vector and its derivatives were used as a system to demonstrate hybridization to short oligonucleotide probes of 6, 7 or 8 nucleotides. To allegedly show the general utility of the proposed conditions, Drmanac examined hybridization of 4 heptamers, 10 octamers and 14 additional probes up to 12 nucleotides long. To allegedly show the utility of the method in fingerprinting unknown clones for the presence of a short sequence, three probes 8 nucleotides long were tested on a collection of 51 plasmid DNA dots made from a library in Bluescript vector. Drmanac concluded that using low-temperature conditions, sufficient difference in hybridization signal was obtained between the dot containing the perfect and mismatched targets and the dot containing only the mismatched targets.

Drmanac does not expressly teach any method for sequencing a target nucleic acid. Instead this reference describes conditions for optimal hybridization of oligonucleotides to cloned DNA. It does not each or suggest elucidating the sequence of a target nucleic acid by determining the molecular weight of any nucleic acid. Drmanac also does not teach or suggest a method of detecting a target nucleic acid that includes a step of determining the molecular weight of any nucleic acid and thus does not teach or suggest the method of claim 77.

Furthermore, Drmanac does not teach or suggest any other embodiments of the claimed methods for sequencing a nucleic acid or the claimed arrays as set forth in the instant dependent claims and outlined above in the discussion of the Khrapko reference.

The Office Action fails to establish that the claims are *prima facie* obvious.

Analysis

The combination of cited references does not result in the instantly claimed methods

Claims 1-55, 58-60, and 63-77, as well as claims 88-123 and claim 128, each require the determination of the molecular weights for nucleic acids of a target array. As discussed above, neither Khrapko nor Drmanac, singly or in any combination thereof, teaches or suggests a method of sequencing that includes a step in which the molecular weight of any nucleic acid is determined. Neither reference suggests that molecular weight determination is necessary for sequence determination, and neither method employs a molecular weight determination step. Thus, the assertion set forth in the Office Action that "a molecular weight [determination] step is implicit in any prior art method of nucleic acid sequencing" is not valid. The primary reference cited in the Office Action, i.e., Khrapko, itself proves that the inaccuracy of the assertion.

In fact, Khrapko in effect teaches away from the use of molecular weight determination in sequencing of nucleic acids. On page 375 of Khrapko it is stated that researchers are seeking and developing new sequencing strategies in order to avoid the disadvantages associated with classical Sanger, and Maxam and Gilbert, sequencing methods. Thus, not only does Khrapko fail to teach a sequencing method that includes a molecular weight determination step, it clearly fails to even suggest or provide any motivation to modify the described methods to include a molecular weight determination. On the contrary, the reference in effect teaches away from such a step. Drmanac does not cure the defects of the Khrapko reference because, as discussed above, it also fails to teach, suggest or provide any motivation for determining the molecular weight of nucleic acids involved in a hybridization reaction. Therefore, the Office Action fails to set forth a prima facie case of obviousness of the claimed methods of sequencing or detecting a target nucleic acid.

Although claims 86 and 87, which are directed to a system containing a

mass spectrometer, a computer and an array of nucleic acid probes, are rejected on the same basis as set forth for the rejection of the claims directed to methods of sequencing a target nucleic acid, it does not appear that such claims are within the purview of the rejection. The systems of claims 86 and 87 are compositions that do not include a "step" of molecular weight determination, and thus it is not clear how a rejection based on the alleged "implicit" molecular weight determination step of all nucleic acid sequencing methods applies to these claims.

As discussed in the Response (mailed April 4, 2001) to the previous Office Action, the combination of teachings of the cited references (Khrapko and Drmanac) provides no suggestion for the preparation of arrays of probes that are immobilized on a solid support that includes matrix material for mass spectrometry (claims 124-126), nor an array that contains 4^R immobilized probes that contain a double-standed portion and a single-stranded portion that includes a variable region of length R (claim 124). Because neither reference mentions or suggests anything about mass spectrometry, there is no teaching or suggestion for inclusion of matrix in the support on which probes are immobilized.

Therefore, the Examiner has failed to set forth a prima facie case of obviousness.

* * *

In view of the amendments and remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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